RUMEN MICROBIAL METABOLISM AND RUMINANT DIGESTION

Editor : J.-P. JOUANY

SCIENCE UPDATE

INRA
EDITIONS

RUMEN MICROBIAL METABOLISM AND RUMINANT DIGESTION

Editor :
J.P. JOUANY

INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE
147, rue de l'Université - 75338 Paris cedex 07

Acknowledgements

The successful completion of this book could not have been achieved without the efficient assistance of Marie-Hélène Farce and the active involvement of Sylvie Toillon.

The editor is indebted to the staff of the Digestion microbienne, Microbiologie, Dynamique de la digestion, Ingestion, Valeur alimentaire, Lactation et Elevage des Ruminants research units and also to Odile Bernard, who played an essential part in the preparation and realization of the summer school project.

ISBN : 2 - 7380 - 0345 - 1

Foreword

By providing us with milk and meat, ruminants fulfil an essential need, but at the same time ruminants are not in direct competition with man for the use of the planet's resources. Ruminant diet is based on roughages that monogastrics, and humans in particular, are unable to use. Moreover, ruminants are closely involved in the balance and upkeep of our environment by grazing natural grasslands and limiting the amount of land left to waste. They also play a part in maintaining economic activity in rural areas, which are becoming increasingly depopulated.

The development of intensive breeding systems over the last twenty years has seen an increased use of feeds rich in energy and high quality proteins. While this practice is possible in developed countries, it can no longer be justified in regions where cereal production is insufficient to meet human food requirements and where the economic situation has worsened because proteins and cereals have had to be imported. The idea of returning to farming systems that make greater use of roughages and which are therefore less intensive, is now gaining ground. It would be one means of combating wasteful overproduction and would allow the ruminant to better fulfil its function in the upkeep of the land. There is now a need therefore for research into how the rumen "fermenter" can best make use of the plant biomass at its disposal.

Ruminants are able to use plant cell wall constituents and non-amino nitrogen through the biotransformation of these substances by rumen microbes into products that can be directly assimilated by the animal. Between 1950 and 1980 much research was done on the bacteria and protozoa that form the basis of the rumen microbial ecosystem. The fungi were discovered only in 1975 but are now studied in numerous laboratories throughout the world. The rapid developments in biotechnology and molecular biology in the last ten years have opened up new areas of research and produced interesting results in the field of rumen microbiology. It was in this context that a group of European researchers (D.I. Demeyer - Belgium, M. Durand and J.P. Jouany - France, and R.A. Prins - Holland) decided to organise a summer school for the training of young post-doctoral research workers. The first course was held in september and october 1990 at the INRA Centre of Clermont-Ferrand/Theix, and a second one is scheduled to take place in 1993. The aims of the summer course were to present latest research developments, to encourage exchanges between young researchers and to create an informal international network of relations including experienced researchers who took part in the course teaching.

This book brings together the texts of the lectures given during the course and of the debates organised at the workshop discussions. It should be of use to university teachers and students alike as well as of interest to researchers. It was thought too difficult to include records of the practical work sessions and the classes dealing with methods and techniques.

There was also a film made, entitled "a journey through the rumen" in which the rumen microbial ecosystem is presented and its interest for the ruminant explained. It describes how plant cell walls are degraded by the rumen microbes and how the ruminants benefit from this process.

J.P. Jouany

Contents

Part one: Microbes in the rumen

Part two: Feed degradation in the rumen and its control

Part three: Modelisation of the rumen

Part four: Reports of workshops

List of contributors

BAUMONT R.
Station de Recherches sur la Nutrition des Herbivores,
Unité Ingestion,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

BESLE J.M.
Station de Recherches sur la Nutrition des Herbivores,
Unité Digestion Microbienne,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

BOHATIER J.
Laboratoire de Protistologie, UA CNRS 138, Université Blaise Pascal,
Complexe scientifique des Cézeaux,
63177 AUBIERE Cedex, FRANCE

CHENOST M.
Station de Recherches sur la Nutrition des Herbivores,
Unité Valeur des Aliments,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

CZERKAWSKI J.W.
6 Elms Way, MAYBOLE, AYRSHIRE,
SCOTLAND, KA19 8BB, UNITED KINGDOM.

DEMEYER D.I.
Research Center for Nutrition, Animal Production and Meat Technology,
University of Ghent, Proefhoeverstraat 10,
9230 MELLE, BELGIUM.

DORÉ J.
Laboratoire de Microbiologie,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

DOREAU M.
Laboratoire de Recherches sur la Lactation et l'Elevage des Ruminants,
Unité Lactation,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

DUCLUZEAU R.
Directeur Scientifique des Productions Animales,
INRA, 147 rue de l'Université,
75338 PARIS cedex 07, FRANCE.

DURAND M.
Laboratoire de Nutrition et Sécurité Alimentaire,
INRA, Centre de Recherches de Jouy-en-Josas,
78352 JOUY EN JOSAS cedex, FRANCE.

FONTY G.
Laboratoire de Microbiologie,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

FORANO E.
Laboratoire de Microbiologie,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

GÄBEL G.
Department of Veterinary Physiology,
Free University of Berlin,
Koserstrasse 20,
1000 BERLIN, GERMANY.

GEAY Y.
Laboratoire de Recherches sur la Croissance et le Métabolisme des Herbivores,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

GOUET P.
Laboratoire de Microbiologie,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

GRENET E.
Station de Recherches sur la Nutrition des Herbivores,
Unité Ingestion,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

HVELPLUND T.
National Institute of Animal Science,
Department of Research in Cattle and Sheep,
Foulum, Postboks 39,
8830 TJELE, DENMARK.

JEAN-BLAIN C.
Département de Pathologie animale,
Ecole Nationale Vétérinaire, B.P. 83,
69280 MARCY L'ETOILE, FRANCE.

JOUANY J.P.
Station de Recherches sur la Nutrition des Herbivores,
Unité Digestion microbienne,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

KAYOULI C.
Chaire de Zootechnie,
43 Avenue Charles Nicolle,
1002 TUNIS BELVEDERE, TUNISIE.

KOMISARCZUK-BONY S.
Station de Recherches sur la Nutrition des Herbivores,
Unité Digestion-microbienne,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.
postal address :
Ecole Nationale Vétérinaire, B.P. 83,
69280 MARCY L'ETOILE cedex, FRANCE.

LEONHARD S.
Department of Veterinary Physiology,
Free University of Berlin,
Koserstrasse 20,
1000 BERLIN, GERMANY.

MALBERT C.H.
Département de Pathologie animale,
Ecole Nationale Vétérinaire,
23 chemin des capelles,
31076 TOULOUSE cedex, FRANCE.

MARTENS H.
Department of Veterinary Physiology,
Free University of Berlin,
Koserstrasse 20,
1000 Berlin, GERMANY.

PONCET C.
Station de Recherches sur la Nutrition des Herbivores,
Unité Dynamique de la Digestion,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

PRINS R.A.
Department of Microbiology, Biology Centre,
University of Grningen, Kerklaan 30,
P.O. BOX 14, 9750 AA, THE NETHERLANDS.

RAMANGASOAVINA B.
Station associée de Nutrition et d'Alimentation,
INRA, INA PG,
16 rue Claude Bernard,
75231 PARIS cedex 05, FRANCE.

RÉMOND B.
Laboratoire de Recherches sur la Lactation et l'Elevage des Ruminants,
Unité Lactation,
INRA, Centre de Recherches de Clermont-Ferrand/Theix,
63122 SAINT-GENES CHAMPANELLE, FRANCE.

SAUVANT D.
Station associée de Nutrition et d'Alimentation,
INRA, INA PG,
16 rue Claude Bernard,
75231 PARIS cedex 05, FRANCE.

STEWART C.S.
Rowett Research Institute,
Greenburn Road,
ABERDEEN AB2 9SB, UNITED KINGDOM.

TAMMINGA S.
Department of Animal Nutrition,
Agriculture University, HAAGSTEEG 4/6708 PM,
WAGENINGEN, THE NETHERLANDS.

THIVEND P.
ENSA,
65 rue de Saint-Brieuc,
35042 RENNES cedex, FRANCE.

TISSERAND J.L.
Laboratoire associé de Recherches en Zootechnie,
INRA - ENSSAA,
26 Boulevard du Docteur Petitjean,
21000 DIJON, FRANCE.

VAN NEVEL C.J.
Research Center for Nutrition, Animal Production and Meat Technology,
University of Ghent, Proefhoeverstraat 10,
9230 MELLE, BELGIUM.

VÉRITÉ R.
Station de Recherches sur la Vache Laitière,
INRA, Domaine de la prise,
Saint-Gilles,
35590 L'HERMITAGE, FRANCE.

WALLACE R.J.
Rowett Research Institute,
Greenburn Road,
ABERDEEN AB2 9SB, UNITED KINGDOM.

WILLIAMS P.E.V.
Colborn-Dawes Nutrition Ltd,
Delves Road, Heanor Gate,
Heanor DE 775 G,
DERBYSHIRE, UNITED KINGDOM.

XANDÉ A.
Station de Recherches zootechniques,
INRA, Centre de Recherches Antilles-Guyane,
Domaine Duclos, Petit bourg B.P. 1232,
97184 POINTE A PITRE cedex,FRANCE.

Introduction

The digestion and nutrition of ruminants are closely linked to the presence of microbes in the animals' rumen. The microbes degrade the food ingested and then ferment the simple molecules that are formed. They use the ATP produced during fermentation to synthetize proteins from simple forms of nitrogen (ammonia for the bacteria or amino acids for the protozoa). The end products of the microbial metabolism are then metabolized by the animal. Thus, volatile fatty acids supply about 70 % of ruminants' energy needs. The microbial cells, which are rich in protein (50 % or more of dry matter), leave the rumen and are digested in the intestine. They provide between 50 and 95 % of amino acids to the animal. The microbes also synthetize vitamins and long chain fatty acids, which are essential to the animals' metabolism. They are also involved in the degradation of certain toxins present in food. The relationship between the ruminant and rumen microbes is therefore symbiotic. If all microbial germs are eliminated from the rumen while the animal is fed on a roughage diet, this one dies.

The rumen microbial ecosystem is complex. It comprises several hundred different species whose populations are highly concentrated (10^4 to 10^{10}). Recent research has shown close interrelations between these micro-organisms. For example, it was observed that for cellulolysis to be efficient in the rumen, there had to be an association of hydrogenotroph species and hydrogen producing species.

Electron microscopic study of plant tissues during digestion and microbial colonization has made it possible to observe and understand the interactions between the micro-organisms and plant cell walls. The technique of image analysis should provide further quantitative information as a complement to these purely qualitative observations.

As research into the rumen has developed, workers have attempted to control its functioning. Thus, feed additives that modify microbial balance or enzyme activities can be used to act on the nature and amount of end products available for the animal. It is now possible to selectively eliminate certain microbes from the rumen (the protozoa can be totally eliminated or a monospecific fauna can be obtained) and thereby to have a better understanding of their role in the ecosystem and even perhaps to control their functioning. Work has recently begun on the characterization of the genes of rumen microbes and certain genes have been cloned and transferred to other micro-organisms. However, to our knowledge, none of these genetically modified genes has been able to develop in the rumen and express its new genetic potential.

Although great progress has been made in our understanding of rumen microbes over the last 20 years, it is also true that the means by which researchers can modify the functioning of the rumen are few. Further studies are needed on the physiology, biochemistry and ecology of these micro-organisms before improvements can be made to rumen efficiency, which is the result of millions of years of natural selection.

All those who have taken part in the preparation of this book are directly involved in active research on rumen function. This is a guarantee for the quality and the actualization of the scientific information given in this book.

PART ONE

Microbes in the rumen

The rumen bacteria

C.S. STEWART

Introduction - The role of rumen bacteria

The rumen bacteria are members of a microbial consortium that performs several functions vital to the well-being of the host animal.

1) Fibre and other polymeric plant material not degraded by host enzymes, is fermented to volatile fatty acids, carbon dioxide and methane. These fatty acids pass through the rumen wall into the circulatory system and are oxidized in the liver, supplying a major part of the energy requirements of the host. Fatty acids may also be directly utilized by the host as building blocks for synthesis of cell material.

2) Fermentation is coupled to microbial growth, and the microbial cell protein synthesized forms the major source of protein for the animal.

3) Rumen microbes synthesize certain vitamins that can be utilized by the host.

4) Some rumen bacteria degrade toxic components of the diet. The best known example is the degradation of the toxic amino acid mimosine and its derivatives, components of the forage legume *Leucaena,* by some rumen bacteria (Allison *et al.*, 1987; Dominguez-Bello and Stewart, 1990a).

The predominant rumen bacteria are strict anaerobes. They are adapted to life in the virtual absence of oxygen, at least as far as its use as a terminal electron acceptor is concerned, but in other respects are as complex as the aerobic procaryotes (Gottschalk, 1981). It is clear that as a result of their adaptation to survival and growth in this environment, the rumen bacteria employ strategies that are largely dictated by conditions in the rumen.

J.P. Jouany ed. : Rumen microbial metabolism and ruminant digestion.
INRA Editions, Paris, 1991, 15-26

1. The rumen environment and its effect on the bacterial population

It is usually thought that conditions in the rumen are very stable. The temperature is around 39°C, very little oxygen is present, in forage-fed animals at least the rumen pH remains reasonably stable and, although meals may be irregular, the microbial population is well adapted for the digestion of feedstuffs of limited use to other animals. In reality, modern feeding regimes may result in rapid fermentation of excess substrate leading to an unstable microflora, acidosis and even death (Hungate, 1966). The rumen may contain toxic compounds, not only of plant origin, but also antibiotics or other substances deliberately added to the feed to reduce methanogenesis. In short, the events in the rumen are in reality more dramatic than our superficial appreciation might suggest, and healthy rumen function depends critically on reactions carried out by micro-organisms.

2. Anaerobiosis

The anaerobic environment prevents the use of O_2 as terminal electron acceptor by the bulk of the microbial population. It has been argued that the microbial population adherent to the rumen wall contains a high proportion of facultative organisms, able to use O_2 that diffuses from the bloodstream (Cheng *et al.,* 1979). Such organisms comprise only a very small part of the rumen bacterial population however, and most rumen bacteria grow under conditions of very low oxygen tension.

Like other micro-organisms, the rumen bacteria conserve energy by substrate-linked and electron-transfer linked phosphorylation of ADP to ATP. The principal substrate-linked phosphorylation reactions are well known and are reviewed elsewhere in this course. Briefly, they involve transfer of phosphate groups from 1, 3 bisphosphoglycerate, phosphoenolpyruvate, acetyl phosphate and butyryl phosphate to ADP by phosphoglycerate-, pyruvate-, acetate- and butyrate kinases (Prins, 1977). Many rumen bacteria employ the first 3 reactions listed, and butyrate producers such as *Butyrivibrio fibrisolvens* possess butyrate kinase.

The rumen environment however imposes constraints on the electron-transfer linked reactions coupled to ATP synthesis. The oxidation/reduction potential (the tendency to gain or lose electrons to another redox couple) of some electron donor or acceptor redox couples in bacteria are shown in Table 1.

Table 1. Standard oxidation/reduction potentials of some redox couples.

Redox couple	E'o (mV)	Redox couple	E'o (mV)
1/2 O_2 / H_2O	+ 820	NO_3^- / NO_2^-	+ 433
Fumarate / succinate	+ 33	SO_4^{2-} / SO_3^{2-}	- 60
NADH / NAD+	- 320	H_2 / H^+	- 420
Lactate / pyruvate	- 197		

In the absence of oxygen, and in the presence of only small amounts of nitrate and sulphate, fumarate/succinate is the most electropositive redox couple available to a number of species of rumen bacteria as an electron acceptor for electron-transport linked phosphorylation. The generation of ATP linked to the reduction of fumarate (fumarate + H_2 = succinate) has been demonstrated in particulate fractions from *Vibrio (Wolinella) succinogenes* (Kroger, 1977). In environments in which nitrates or sulphates are present at high concentrations, the reduction of these compounds competes for reducing equivalents with the reduction of CO_2 to CH_4 (Gibson *et al.*, 1990). However, the concentrations of nitrates and sulphates in the rumen are low compared to the concentration of CO_2, and although nitrates and sulphates are readily reduced, methanogenesis is a quantitatively more important means of electron disposal.

3. Methanogenesis

The presence of methanogens in the rumen has a profound effect on the fate of organic carbon and hydrogen during fermentation (reviewed by Wolin and Miller, 1988). Although a number of bacterial species can utilize H_2 when grown in pure culture (Henderson, 1980), methanogens possess hydrogenases that bind H_2 at very low concentrations. Hungate *et al.*, (1970) reported that the Km for H_2 of the *Methanobacterium ruminantium* hydrogenase was around 1 x 10^{-6} M, demonstrating between 4 and 40 times greater affinity for H_2 than the propionate/succinate producing rumen bacteria tested by Henderson (1980). The interspecies transfer of H_2 to methanogens results in competition for reducing equivalents with other reactions, such as the reduction of pyruvate to lactate or ethanol, and increased flow of substrate C to acetate (summarized schematically in Figure 1).

Since the formation of acetate from acetyl CoA via acetyl kinase is linked to formation of ATP (above), the presence of an H_2 sink in the form of methanogenesis

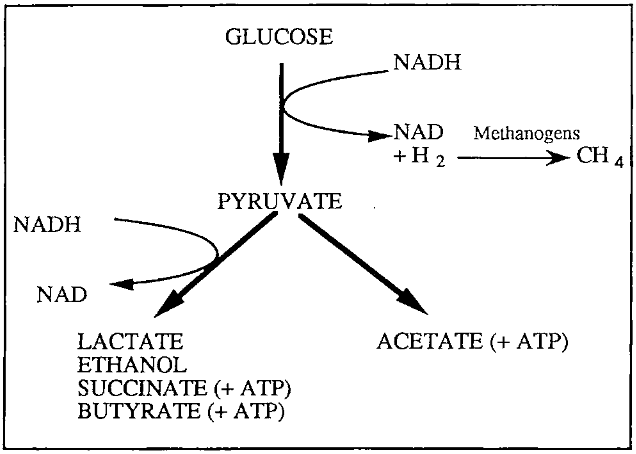

Figure 1. Fate of hydrogen in some fermentations. In the presence of methanogens, NADH is reoxidized by the formation of H_2, and acetate is formed in preference to reduced products such as lactate, ethanol, etc...

can be energetically favourable for those organisms like *Ruminococcus albus* that produce acetate and H_2 together with lactate and/or ethanol in axenic culture (Wolin and Miller, 1988).

4. Residence time of substrates in the rumen

The flow of digesta through the rumen, with liquid turnover around every 12 h and solid retention times of (normally) 48 h or less, imposes a constraint for some reactions. Some very slow reactions, such as the formation of methane from acetate, do not occur to a significant extent in the rumen. Furthermore, the residence time of cellulosic dietary material can be an important factor limiting digestibility in the rumen (Blaxter *et al.,* 1956; Hungate, 1975), though small particles may be further degraded in the hindgut.

5. Toxic compounds and potential uncouplers

In addition to plant compounds toxic to animals such as mimosine (above; reviewed by Dawson and Allison, 1988) a number of compounds potentially toxic to micro-organisms are present in the rumen. Volatile fatty acids in their undissociated form are toxic to many enteric bacteria, but rumen bacteria are relatively resistant to these compounds at pH values within the rumen range (Stewart, 1975). Some plant phenolic acids have an antimicrobial activity (Chesson *et al.,* 1982), and it is thought that coumarin (1,2 benzopyrone) and other substituted phenolic compounds of plant origin may act as uncoupling agents (Cansunar *et al.,* 1990). The arginine analogue canavanine, a component of the legume *Canavalia ensiformis,* has been shown to inhibit growth of some rumen bacteria, though other rumen bacteria degrade canavanine (Dominguez-Bello and Stewart, 1990b).

6. Fluctuations in substrate supply

It is assumed that in grazing and browsing ruminants consuming forage, the fermentation is limited by the relatively slow breakdown of the polymers (cellulose and hemicelluloses) that comprise the major sources of energy for the growth of rumen micro-organisms. Under these conditions, the adaptation of the rumen microflora encourages the growth of species like *Bacteroides, Selenomonas, Ruminococcus* and others that are able to maintain high growth yields by synthesizing 3 or more moles ATP per mole of substrate fermented. In the case of *Ruminococcus albus* which appears to lack opportunities for electron transport-linked phosphorylation, high growth yields are dependent on the presence of methanogens as discussed earlier. In practice even wild ruminants grazing lush pasture do not maintain continuous feed intake, and the bacteria in the rumen have to adapt to periods of substrate deficiency. Many of the rumen bacteria store excess sugar intracellularly in the form of glycogen granules (Hungate, 1966), which can be utilized as an energy source during such periods.